

- Supporting Information -

Unambiguous Phosphosite Localization using Electron-Transfer / Higher-Energy Collision Dissociation (EThcD)

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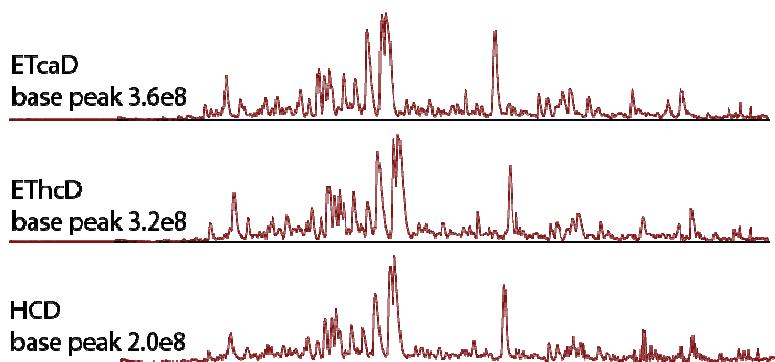
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Supplementary table 1: overview of phosphopeptide sequences in the primeXS mixture used in this study. a) peptide identifier. b) peptide sequence. Red letters indicate phosphorylated residue. c) position of phosphorylation. d) number of PSM (phosphosite correctly annotated by SEQUEST/identified). e) average SEQUEST Xcorrs.

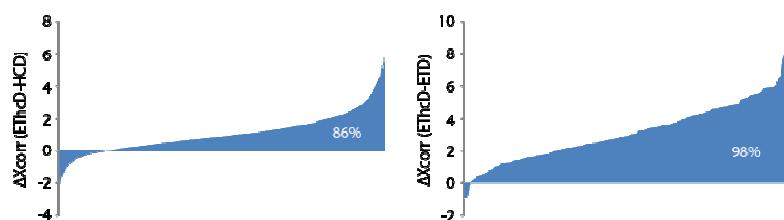
primeXS # ^a	phosphopeptide sequence ^b	phosphosite ^c	number of PSM ^d			average SEQUEST Xcorr's ^e		
			ETD	HCD	EThcD	ETD	HCD	EThcD
1	NSVEQGRRL	S2			16/16			1.9
2	APPDNLPSPGGSR	S12	18/22	18/26	24/30	0.7	1.2	2.0
3	LIEDNEYTAR	Y7		20/22	26/26		2.3	2.4
4	RS ^D GGHTVLH ^R	S2	1/1	1/1	2/2	2.3	2.3	2.6
5	ENIMRSSENESQLTSK	S6	11/11	11/11	6/9	1.7	3.1	3.8
6	QLGEPEK ^S QDSSPVLSELK	S8, S16	1/4	7/8	3/3	1.5	2.1	4.4
7	QLGEPEK ^S QDSSPVLSELK	S8, S11, S16	1/4	2/3	2/2	1.3	2.1	4.5
8	KFL ^S LASNPPELLNLPPSSVIK	S4, S17						
9	THILLFLPK ^S VSDYEGK	S10						
10	WWGSGPSGSGGS ^G GGGK	S12	6/8	4/9	9/9	0.9	1.2	2.5
11	WWGSGPSGSGGS ^G GGGK	S9, S12	1/5	0/7	8/9	0.8	1.0	2.6
12	WWGSGPS ^G SGSGS ^G GGGK	S7, S9, S12	1/2	2/4	4/4	0.8	0.7	1.9
13	WWGSGPSGSGGS ^G GGGK	S4, S7, S9, S12						
14	GSRAAK ^T LGS ^R LGK	S2, T7, S10	41/41	32/32	35/35	2.1	0.9	2.2
15	RYRVAKL ^G SSLGK	Y2, S9, S10	31/31	18/18	3/3	1.5	1.4	2.1
16	RSRVVKLGSS ^S LGK	S2, S9, S10, S11	22/22	4/4		1.4	1.5	
18	SAG ^S R ^T L ^S GR	S4, T7, S9	6/6		10/12	1.9		2.0
19	LQLQGLDL ^S SR	S9, S10	3/3	6/6	5/5	0.6	0.8	1.3
20	LEKT ^I DDELDK	T4	10/10	14/14	16/16	1.3	2.1	2.9
21	RGDDSF ^G D ^K	S5			1/1			2.2
22	GSAHGSGYSGASGHSGAGSGGK	S2	3/3	1/1	2/2	2.1	4.1	2.9
23	GSAHGSGYSGASGHSGAGSGGK	S2, S6	6/7	7/7	5/5	2.2	3.6	3.0
24	GSAHGSGY ^S SGASGHSGAGSGGK	S2, S6, S9	5/6	1/2		2.1	2.5	
25	GSAHGSGYSGASGHSGAGSGGK	S2, S6, S9, S12	1/3			1.6		
26	LQLQGLDL ^S SR	S9		4/7	8/8		0.9	2.0
27	TYET ^T LEK	T1, Y2, T4			3/3			1.9
28	VPQVSTPTLVEVSR	S5, T6, T8		5/5	7/7		1.5	1.4
29	TASDTDSSYAIPTAGMSPSR	Y9	5/6	12/14	10/13	1.4	3.1	3.1
30	SVENLPEAGI ^T HEQR	T11	17/17	22/22	11/11	1.3	2.6	2.6
31	FGSSNTDSAGALG ^T LR	S3, S4, T14	4/4	3/14	15/17	1.6	1.6	2.8

Supplementary figure 1: Ti4+-IMAC enriched phosphopeptide analysis. A) Base peak chromatograms highlight the reproducibility of the three individual LC-MS/MS runs. B) SEQUEST Δ Xcorr values show that EThcD provides higher quality MS/MS spectra which leads to higher SEQUEST scores.

A)



B)



Associated content

The data associated with this manuscript may be downloaded from ProteomeCommons.org Tranche using the following hashes and passwords:

synthetic phosphopeptides dataset

z3d+D0DTM34eaR+OnKOATIXH5V5FcamYRixhLrgEYJKyme7008M9B1dTa0vja03XXIp7PcaxOVw3owJ2jn/f
3JwweH0AAAAAAAAGiA==

password: HIw9hraMQiQRHNhgK4Kp

Ti-IMAC phosphopeptides dataset

or4XwxucZhCTxtsKCPA1VvVRIWtJzRoiObPRQ47I5ashRMWJoQ4idydEh3yLlmiyv3LQXnX9fNuKpKF48xLs9
bISdLUAAAAAAADvg==

password: fcfiv7lEP2qemhZCyzbg